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学位論文題名	Diversity and depth-dependent distribution of bacteria within hot spring-associated phototrophic microbial mats using 16S rRNA gene amplicon analyses and metagenomic insights 16S rRNA 遺伝子アンプリコン解析とメタゲノム解析に基づく温泉光合成微生物被膜のバクテリアの多様性と深度依存（英文）
論文審査委員	主査　教授　花田　智 委員　教授　春田　伸 委員　准教授　得平　茂樹 委員　准教授　Thiel, Vera

【論文の内容の要旨】

Phototrophic mats are microbial assemblages dominated by photosynthetic microbes and develop in various light-exposed environments such as hot springs, lakes and hypersaline waters. In an elevated temperature in hot springs, microbial key players are low in variety, making these mats suitable model system for studying microbial diversity and population ecology in photosynthesis-driven communities.

Phototrophic microbial mats in a sulfidic and slightly alkaline hot spring, Nakabusa Hot Springs (Nagano, Japan) have been vigorously studied for the last decades. In this study, a culture-independent method using next-generation sequencing was employed in order to determine the composition and vertical distribution of bacteria and to estimate the metabolic functions of the most abundant, putatively novel microbial community members of the mat developed at approximately 56-64°C spring water.

Microbial mat samples (~3-5 mm) were collected over two years (6 samples) and five layered sections (approx. 1 mm each) were prepared. Genomic DNA extraction and 16S rRNA amplicon sequencing were carried out to disclose the microbial diversity of the mats. Metagenome sequencing was done to predict their metabolic potentials. 16S

rRNA amplicon sequencing indicated the existence of a high diversity of phototrophs representing the phyla *Cyanobacteria, Chloroflexi, Chlorobi*, *Proteobacteria*, and *Acidobacteria*. A stable core community could be identified and predominated (44% relative abundance of sequence reads) by cyanobacteria and filamentous anoxygenic phototrophs (FAPs) in the phylum *Chloroflexi*. The vertical distribution of the phototrophic and chemotrophic members among five layers of the mat was likely well-correlated with the spectral irradiance and oxygen concentrations over depths. In the upper mat layers, a high abundance of cyanobacteria (*Thermosynechococcus* sp.) was detected as expected from the strong absorbance spectrum of chlorophyll and phycobiliprotein in the mat surface. The FAPs, *Roseiflexus castenholzii* and *Chloroflexus aggregans,* could correlate together with spectral absorption of BChl *a* and *c* penetrating in the deeper mat layers.

Metagenome binning (classifying different members) retrieved 23 partial genomes of which two representing abundant chemotrophs were analyzed in detail. The partial genomes of these two abundant unidentified members of the mat possessed no photosynthetic genes. One of them was phylogenetically belonging to unidentified *Acidobacteria*. The decreasing relative abundance of this member into the bottom layers indicates aerobic metabolism. The other non-phototroph, a novel putative sulfate-reducing bacterium, designated to be *Ca.* Thermonerobacter sp. increased in abundance over depth enabling metabolism in the anaerobic niche in the deeper layers.

Overall, the present study found a high diversity of phototrophs in the microbial mats and clarified the depth-dependent distribution of microbial members including novel species. Metagenomic approach could estimate the metabolic properties of the unknown members. Niche differentiation along with the microenvironmental gradients of light and oxygen probably allow their coexistence and efficient metabolic exchanges in the microbial ecosystems.